June 13, 2008

Mr. Jim McKenna
Port of Portland & Co-Chairman, Lower Willamette Group
121 NW Everett
Portland, Oregon 97209

Mr. Robert Wyatt
Northwest Natural & Co-Chairman, Lower Willamette Group
220 Northwest Second Avenue
Portland, Oregon 97209

Re: Portland Harbor Superfund Site; Administrative Order on Consent for Remedial
      Investigation and Feasibility Study; Docket No. CERCLA-10-2001-0240.
      Toxicity Reference Values Methodology – Aquatic Biota Tissue

Dear Messrs. Wyatt and McKenna:

        On April 11, 2008 EPA provided the recommended Toxicity Reference Values
(TRVs) for the water, sediment and dietary assessment of fish and wildlife to be used in the Portland
Harbor baseline ecological risk assessment (BERA). In that letter EPA advised the LWG that tissue-
residue TRVs were still under development.   EPA has also advised LWG that without a
compendium of baseline ecological risk assessment aquatic biota tissue TRVs from which tissue
TRVs for the Portland Harbor BERA can be selected, a TRV derivation methodology or
hierarchy will have to be defined. The purpose of this letter is to provide EPA's methodology for
derivation of aquatic biota tissue TRVs.

        EPA has identified two primary approaches using the existing scientific literature to
derive baseline ecological risk assessment aquatic biota tissue TRVs as the most appropriate for
aquatic biota tissue TRV derivation at Portland Harbor. The two baseline tissue TRV derivation
approaches EPA is recommending are the Species Sensitivity Distribution (SSD) approach and
the Lowest Value approach.   The attached methodology describes the approaches, presents a
hierarchy of procedures to be used to develop TRVs, including selection of the specific approach
to be used depending on data availability, and a list of chemicals for which aquatic biota tissue
TRVs need to be derived.

The list of chemicals for which tissue TRVs need to be derived for the BERA is based on the tissue sample results available through the completion of the Round 2 data report. We anticipate that TRVs for few, if any additional chemicals will be required after tissue data collected as part of the Round 3 sampling events are screened.

EPA's desired outcome from the aquatic biota tissue TRV derivation process is to develop TRVs that are based on measured tissue residues from various aquatic species that are associated with adverse ecological effects or unacceptable ecological risks to the assessment endpoints for various categories of ecological receptors at Portland Harbor. EPA is currently using the attached methodology to derive tissue TRVs using the residue-effects information EPA and LWG have previously shared with each other. We will provide you with the numerical values of the TRVs and the data that were used to derive the TRVs as soon as the TRV derivation is complete.

If you have any questions, please contact Chip Humphrey at (503) 326-2678 or Eric Blischke (503) 326-4006. All legal inquiries should be directed to Lori Cora at (206) 553-1115.

Sincerely,

Chip Humphrey
Eric Blischke
Remedial Project Managers

cc: Greg Ulirsch, ATSDR
        Rob Neely, NOAA
        Ted Buerger, US Fish and Wildlife Service
        Preston Sleeger, Department of Interior
        Jim Anderson, DEQ
        Kurt Burkholder, Oregon DOJ
        David Farrer, Oregon Environmental Health Assessment Program
        Rick Keppler, Oregon Department of Fish and Wildlife
        Michael Karnosh, Confederated Tribes of Grand Ronde
        Tom Downey, Confederated Tribes of Siletz
        Audie Huber, Confederated Tribes of Umatilla
        Brian Cunninghame, Confederated Tribes of Warm Springs
        Erin Madden, Nez Perce Tribe
        Rose Longoria, Confederated Tribes of Yakama Nation

Aquatic Biota Tissue TRV Derivation

Introduction

Unlike the case where several published sources of screening level aquatic biota tissue benchmarks are available (e.g. Dyer et al. 2000, Shephard 1998), EPA is unaware of any published source of widely available aquatic biota tissue TRVs for use in baseline ecological risk assessments. This means that the aquatic biota tissue TRVs for the BERA will have to be derived from the original residue-effects literature. The two primary compendia of residue-effects literature for aquatic species are the Environmental Residue Effects Database (ERED), found online at http://el.erdc.usace.army.mil/ered/, and the review by Jarvinen and Ankley (1999), most of which was later incorporated into the ERED. EPA has also shared with the LWG an updated (January 2008) version of the residue-effects database described in Shephard (1998), which was the original source for most of the literature originally incorporated in the ERED. Combined with additional studies identified by LWG and other interested parties during the Portland Harbor RI/FS process, these data sources will be used to obtain studies to be used during TRV derivation. All of these sources contain primarily whole body residue-effects information.

Without a compendium of baseline ecological risk assessment aquatic biota tissue TRVs from which tissue TRVs for the Portland Harbor BERA can be selected, a TRV derivation methodology or hierarchy will have to be defined. The primary purpose of any baseline ecological risk assessment at a Superfund site is to determine risks associated with current site conditions, and to assist risk management decisions regarding the need for site remediation. Given that the tissue TRVs will be used in a baseline ecological risk assessment, EPA believes that the TRV derivation methodology must be consistent with EPA's ecological risk assessment paradigm. A paradigm is a philosophical and theoretical framework of a scientific discipline. As such, it differs from a specific protocol or guidance. The intent of the EPA (1997) ecological risk assessment paradigm is to provide a general conceptual framework for organizing problems and risk assessment approaches. Consistency of the tissue TRV derivation methodology with the EPA risk assessment paradigm was a major consideration in EPA's selection of a TRV derivation methodology.

EPA's desired outcome from the aquatic biota tissue TRV derivation process is to develop TRVs that are based on measured tissue residues from various aquatic species that are associated with adverse ecological effects or unacceptable ecological risks to the assessment endpoints for various categories of ecological receptors at Portland Harbor. This is consistent with EPA (1997) ecological risk assessment guidance, which calls for BERA risk characterizations to identify thresholds for effects on the assessment endpoints as a range between contamination levels identified as posing no ecological risk and the lowest contamination levels identified as likely to produce adverse ecological effects. To meet this goal, the tissue TRVs to be derived for the BERA will be LOER (lowest observed effect residue) based, and thus will likely be higher than the screening level benchmarks used in the SLERA.

Derivation Methodologies for Aquatic Biota Tissue TRVs

EPA has identified two primary approaches using the existing scientific literature to derive baseline ecological risk assessment aquatic biota tissue TRVs. Although other approaches may be available, EPA suggests that the following two approaches are the most appropriate for aquatic biota tissue TRV derivation at Portland Harbor. These were selected based on increasing data availability, complexity of calculation, TRV reliability, consistency with the ecological risk assessment paradigm, and ecological realism. Therefore, the two baseline tissue TRV derivation approaches EPA is recommending are:

1. Lowest Value Approach
2. Species Sensitivity Distribution (SSD) approach.

Both of these methodologies will be used to derive tissue TRVs for the Portland Harbor BERA. The strengths, weaknesses and application of these two tissue TRV derivation approaches in the BERA are discussed in the following section. Given that there are no available compendia of aquatic biota tissue TRVs for use in baseline ecological risk assessments for Superfund, EPA believes an extended discussion of both methodologies is necessary to justify the hierarchy of TRV derivation methods presented at the end of this section.

Lowest Value Approach

The lowest value approach evaluates all available toxicity data for a contaminant. After the data are compiled, the lowest relevant toxicity value (i.e. the lowest residue-effects LOER [lowest observed effect residue] concentration) is selected as the TRV. So long as the LOER is based on an acceptable endpoint for an appropriate species, no further adjustments to the value may be required. However, the LOER may also have to be divided by one or more uncertainty factors to obtain the final TRV. Although many types of uncertainty factors can be considered, the factors applied to the literature-based LOER generally fall into one of three broad categories:

- Acute to chronic adjustment
- Interspecies extrapolation
- Laboratory to field extrapolation

Most residue-effects literature associates a measured residue with reductions in survival using acute (i.e., short-term) exposure periods. Although survival is part of most assessment endpoints in the Portland Harbor BERA, TRVs are often based on reproduction and growth to ensure they are appropriately protective of the most sensitive portion of the assessment endpoint. An uncertainty factor can thus be applied to a literature-based mortality LOER to convert an acute mortality LOER into a LOER or NOER for effects on reproduction and growth. This acute to chronic uncertainty factor (more commonly called an acute-chronic ratio or ACR) is applied because concentrations required to elicit acute mortality are generally higher than the concentrations that reduce growth and/or reproduction.

Unless a species specific acute to chronic ratio is available for residues in the particular study under review, a default acute to chronic ratio is required for TRV derivation. The default acute to chronic ratio for use in the Portland Harbor BERA tissue TRV derivation will be 8.3, based on

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the study of Raimondo et al. (2007). The Raimondo et al. (2007) study is the geometric mean acute-chronic ratio of 456 same-species pairs of acute and maximum acceptable toxicant concentrations for metals, narcotics, pesticides, and other organic chemicals. The default 8.3 acute to chronic ratio (uncertainty factor) will only be applied to LOER values for which mortality was the measured toxicological endpoint. All mortality LOERs will be divided by 8.3 before further use in tissue TRV derivation.

This uncertainty factor is, strictly speaking, most appropriate for use with acute (i.e., relatively short study exposure times) mortality as opposed to chronic (i.e., relatively long study exposure times) mortality. However, most of the tissue toxicity literature studies in which mortality was measured used acute exposure periods, using as the definition of acute an exposure duration less than 10% of the lifespan of the test organism (Rand 1980). The 8.3 uncertainty factor will be applied to all mortality LOERs.

Interspecies extrapolations and laboratory to field uncertainty factor both account for the assumption that laboratory studies underestimate adverse effect concentrations in the field. Reasons for applying an interspecies uncertainty factor include the life stage tested in the laboratory may be less sensitive than another life stage; laboratory test species are often selected because of their ease of handling and culture in the laboratory, and are not representative of the taxonomic diversity found in the field; and concerns that commonly uses laboratory test species may be more tolerant to contamination than are other species. Concerns regarding the use of interspecies extrapolation and laboratory to field uncertainty factors include the possibility that laboratory species and/or test conditions overestimate toxicity under field exposure conditions. Since the objective of tissue TRV derivation for the BERA is to derive a LOER based TRV, interspecies extrapolation and laboratory to field uncertainty factors will not be used during the derivation of Portland Harbor BERA tissue TRVs.

Concerns regarding the scientific basis and validity of the uncertainty factors include the magnitude of the factors, and whether or not the approach is consistent with the risk assessment paradigm. Specific criticisms include the often arbitrary nature of uncertainty factors, their largely empirical nature, and their lack of a theoretical scientific basis (Chapman et al. 1998, Rand et al. 1995, Calabrese and Baldwin 1993). The absence of universally accepted values for uncertainty factors (Calabrese and Baldwin 1993) confirms their often arbitrary nature.

As described herein, the lowest value approach ignores all data except the lowest effect concentration (i.e. the most conservative or worst case approach). This type of approach is more appropriate for a screening level benchmark as opposed to a baseline ecological risk assessment TRV. Ideally, a TRV used within a BERA is developed from multiple acceptable studies, which if desired permits estimation of the probability of risk or the probability of an adverse toxicological effect at a given exposure concentration. Ultimately, uncertainty factors applied to TRV derivation are used to address a lack of knowledge regarding the toxicity of a chemical. Use of the lowest value approach would require that, in addition to agreement on the toxicity value and study used to derive the TRV, agreement would have to be reached on the values of the uncertainty factors to be applied during BERA TRV development.

Given the amount of residue-effects literature available describing the effects of many bioaccumulated chemicals to aquatic life, EPA believes better approaches are available to derive aquatic biota tissue TRVs for the Portland Harbor BERA than the use of the lowest value approach. However, for chemicals with an insufficient amount of residue-effects literature to permit TRV derivation by other methods described in this section, EPA will use the lowest value approach as the last (lowest) rung on the hierarchy of TRV development methods acceptable for use in the Portland Harbor BERA.

The strengths and weaknesses of the lowest value approach to aquatic biota tissue TRV derivation are as follows:

- *Strengths*
    - Simplicity of use
    - Ease of understanding
    - Minimal data requirements - as little as one toxicity value needed to derive a TRV
    - Uncertainty factors, if needed, become larger as toxicity data become more unreliable or uncertain, or if fewer studies are available
    - The magnitude of the uncertainty factor, if needed, can be changed as new toxicological information becomes available

- *Weaknesses*
    - Largely empirical, no theoretical basis
    - Questions regarding the validity of acute to chronic ratios
    - Questions regarding the magnitude of the acute to chronic ratio
    - Not fully consistent with the risk assessment paradigm
    - Lack of transparency – the lowest value approach does not provide a consistent degree of protection to ecological receptors, and thus does not permit informed discussions between risk managers and other interested parties regarding the level of protection occurring

The specifics of the lowest value approach as applied to BERA tissue TRV derivation for Portland Harbor are presented in the aquatic biota tissue TRV derivation methodology presented later in this section.

Species Sensitivity Distribution (SSD) Approach

A species sensitivity distribution is a statistical model which calculates a chemical concentration protective of a predetermined proportion or percentage of a group of species from a defined adverse toxicological effect. In theory, SSDs are intended to provide an indication of both the total range and distribution of species sensitivities in natural communities, even when the actual range of sensitivities is unknown (Stephan 2002). In practice, SSDs are most commonly presented as a cumulative distribution function (CDF) of the toxicity of a chemical to a group of laboratory test species. Perhaps the best known application of SSDs to develop TRVs for ecological risk assessment is their use to derive EPA's ambient water quality criteria (AWQC) for the protection of aquatic life (Stephan et al., 1985).

The general approach to derive a SSD is to obtain the toxicity data for a number of species. In instances where multiple studies have evaluated the same toxicological endpoint on the same species, the data must undergo some preprocessing before it is incorporated into the SSD. Preprocessing procedures to be applied to TRV development for Portland Harbor will be given later in this section.

Several statistical models have been used to fit toxicity data to species sensitivity distributions. These include log-triangular distributions (Stephan et al. 1985), log-logistic distributions (Aldenberg and Slob 1993), lognormal distributions (Wagner and Lokke 1991) and Burr Type III distributions (Shao 2000). There is no known theoretical reason why a SSD for any given data set should conform to a specific statistical distribution. For example, most new approaches for water quality criteria derivation outside of the U.S. select specific SSD derivation models based on which best fit the underlying data distribution from a statistical point of view.

The largest single difference between the various published approaches to deriving SSDs is the statistical distribution fit to the toxicity data. In general, development of an SSD from toxicity data is as follows:

Each data point within an SSD is given equal weighting, i.e. no single study carries more weight within the SSD than does any other study. The SSD is calculated from a cumulative distribution frequency of the species sensitivity to contaminant data by ranking the effect concentration for each species from lowest to highest. The cumulative frequency value for each data point is calculated from Equation 1:

Equation 1:

$$Cumulative\ frequency = Rank \times \left(\frac{100}{n+1} \right)$$

Where:
      n = number of data points used to develop the SSD

The cumulative frequency value (sometimes termed the potentially affected fraction of species) of each data point is then plotted against the effect concentration that represents the sensitivity of that species to the contaminant, yielding the typically S-shaped species sensitivity distribution plot with effect concentrations on the x-axis and the cumulative frequency values plotted on the y-axis.

Regardless of the statistical distribution used to fit the SSD (e.g. log-logistic, lognormal, etc.), the equation describing the distribution is known. This knowledge permits calculation of the concentration protective of any selected proportion of species. The level of protection selected is not a technical or statistical decision, instead, it is ultimately a management decision. The two most commonly used protection percentiles are protection of 95% of all tested species (e.g. Stephan et al. 1985) and 90% of all tested species (e.g. Meador et al. 2002). To afford protection

to these proportion of species, the TRV derived from the SSD is set at either the 5th or 10th percentile of the adverse effect concentrations.

All species sensitivity distributions make a series of assumptions, both statistical and biological. Statistical assumptions generally entail the suitability of the distribution used to fit the SSD, and the number of samples within the SSD, which relates to the reliability and stability of the TRV derived from the SSD. This is particularly true of TRVs selected from a tail of the SSD, where the TRV is lower than all but 5% or 10% of the effects data. Biological assumptions about the SSD approach include: whether communities and ecosystems are sufficiently protected by an SSD-derived TRV intended to protect a defined proportion of species within the community or ecosystem; whether a SSD based on laboratory generated toxicity data yields the same distribution of species sensitivity observed in field situations (i.e. the species incorporated into the SSD are representative of the sensitivities of all species); and whether TRVs derived from SSDs are inherently protective of communities and ecosystems. As described in detail in Posthuma et al. (2002), many of the statistical and biological questions regarding the use of SSDs have been satisfactorily answered to the point where SSDs have been used by a number of regulatory agencies in North America (both the U.S. and Canada), Europe, Asia and Australia to derive environmental quality guidelines.

Within the context of aquatic biota tissue TRV derivation for Portland Harbor, perhaps the two most critical decisions are the minimum number of data points to be used during SSD development, and the level of protection provided by the TRV. The previously published tissue residue benchmarks used for screening in ecological risk assessments (e.g. Dyer et al. 2000, Meador et al. 2002) were derived using whole body lowest observed effect residue (LOER) data, which is the same adverse effect residue data that will be used to derive the Portland Harbor BERA TRVs. The minimum number of samples used to derive an SSD for use in regulatory programs has varied from four (Netherlands environmental risk limits), five (Australia and New Zealand water quality guidelines), eight (USEPA ambient water quality criteria) or 10 (European Union water quality guidelines). Several investigations of the number of data points needed to derive TRVs from SSDs have been performed, including Wheeler et al. 2002, Newman et al. 2000, and Roman et al. 1999. Both Wheeler et al. 2002 and Newman et al. 2000 indicated that relatively sizable data sets (between 10 and 55 data points, depending on the distribution and spread of the data) were required for a highly protective percentile TRV to be stable irregardless of the data set from which the SSD was developed.

Roman et al. (1999) concluded that with fewer than five data points, the lowest value approach (termed the assessment factor approach in their paper) is more precise than the SSD approach, but that increasingly lower TRVs may be generated from the lowest value approach as the number of toxicity studies increases. With five or more data points, the SSD approach for generating TRVs is more consistent with the risk assessment paradigm, as it yields a stable value for the TRV with increasing confidence in the reliability and protectiveness of the TRV as the amount of toxicity data used to develop the SSD increases. The protectiveness of the SSD approach in deriving TRVs has been validated by studies such as Okkerman et al. (1993), who evaluated toxicity based on studies with multiple species exposed to organic chemicals.

Based on a consideration of the literature describing the minimum number of data points required to derive an SSD, EPA recommends that a minimum of five data points be used to derive aquatic biota tissue TRVs for chemicals in the Portland Harbor BERA. Furthermore, EPA will set the level of protection of the tissue TRVs at the 5th percentile for target aquatic ecological receptors to be evaluated at the organism level (i.e., these TRVs would be use both for juvenile salmonids and Pacific lamprey ammocoetes), and at the 10th percentile for all other aquatic biota tissue measurement endpoints which are evaluated at the population level.

The selection of these two percentiles is based on several precedents in the field of ecotoxicology. Most applicable to tissue TRV derivation may be the approach of Meador et al. (2002), who developed a species sensitivity distribution for PCB tissue residues which, if not exceeded in juvenile salmonids, are likely protective of ESA listed species from any adverse effects that may jeopardize the population's ability to recover and increase to sustainable levels. This was defined by Meador et al. (2002) as a residue protective against adverse effects on the ability of individual salmon to grow and mature normally. Meador et al. (2002) concluded that a low percentile of all listed residue-effect studies was an appropriate benchmark for protecting individual juvenile salmonids from sublethal effects that could decrease their long term survival. The PCB residue considered protective against biological effects in migrating juvenile salmonids was chosen as the 10th percentile of the 15 residue-effect concentrations identified by Meador et al. (2002).

The approach used by Meador et al. (2002) of calculating a TRV from a low percentile of a series of rank-ordered residue-effect concentrations is similar to the approach used by EPA (Stephan et al. 1985) to derive ambient water quality criteria. EPA's criterion maximum concentration (CMC, commonly called the acute criterion) is derived from the 5th percentile of an SSD for aquatic genera generated from acute toxicity data. Similarly, the criterion continuous concentration (CCC, commonly called the chronic criterion), can be derived from the 5th percentile of an SSD for aquatic genera derived from chronic toxicity data. More often, the CCC is calculated as the final acute value divided by the final acute-chronic ratio (ACR). The final ACR is based upon chronic values calculated from maximum acceptable toxicant concentrations (MATC) for at least three different species. The MATC is generally considered an estimate of a toxic threshold concentration within the range bounded by a NOEC and a LOEC, and is often considered the highest safe or no effect concentration (Cooney 1995).

ESA listed aquatic species as a group are generally not believed to be more sensitive to chemicals than aquatic species as a whole (Dwyer et al. 2005, Sappington et al. 2001, Dwyer et al. 1999). Based on measured toxicity data with threatened and endangered aquatic species, water quality criteria derived from the 5th percentile of an SSD are therefore generally protective of ESA listed species. In order to further ensure that the 5th percentile of an SSD are protective of ESA and other species to be evaluated at the individual organism level, a final check of the derived TRV will be performed.

The SSD approach has the advantage of previous use by EPA and other regulatory agencies during the development of ecological risk assessment TRVs (e.g. water quality criteria). It also has advantages over the lowest value approach in that the SSD approach uses more information from multiple studies to derive a TRV, has an explicitly defined level of protection, has well

developed statistical and computational procedures available, and has been validated to some extent as being protective of ecological receptors.

The strengths and weaknesses of the species sensitivity distribution approach to aquatic biota tissue TRV derivation are as follows:

- *Strengths*
  - Use toxicity data from all species for which data are available, consistent with the risk assessment paradigm
  - Based on sound statistical procedures, assuming the underlying assumptions of the method are met
  - Flexible, applicable to both risk assessment and risk management
  - Can be derived from any toxicological effect (e.g. survival, reproduction, etc.) or endpoint (e.g. LC_{50}, EC_{20}, LOEC, NOEC etc.)
  - Allow any level of protection desired to be selected except for 0% and 100%
  - Approach is transparent, and allows informed discussions to take place regarding the desired level of protection
  - Can be used in backwards calculations to estimate the level of protection when the contaminant occurs at a specified concentration in the environment
  - Some statistical and biological attributes of the approach have been validated

- *Weaknesses*
  - Minimum data requirements more extensive than other TRV derivation approaches, may limit the number of chemicals for which TRVs can be developed
  - More complex mathematical derivation of TRVs than other approaches
  - Statistical assumptions of SSD derivation may be violated
  - Communities and ecosystems may not be sufficiently protected based on an SSD protecting a given percentage of the species within the community or ecosystem

Minimum Data Requirements, Data Preprocessing and Inclusion Procedures for Aquatic Biota Tissue TRV Development

Not all of the available residue-effects literature contains data suitable for deriving a TRV for use in the Portland Harbor BERA. The selection of studies suitable for TRV derivation generally followed the procedures described in the LWG (2004) *Technical Memorandum: Provisional Toxicity Reference Value Selection for the Portland Harbor Preliminary Ecological Risk Assessment*. The primary requirement is to use studies in which measured whole body residue concentrations are reported to be associated with relevant effect endpoints, defined as effects on survival, reproduction, growth and behavior (LWG 2004). Residues in all life stages of aquatic species, including eggs, are considered. Various exposure routes are considered: dietary, waterborne, and maternal transfer to eggs. Injection and gavage studies were also considered during TRV derivation in LWG (2004). For the Portland Harbor BERA, injection and gavage are not considered to be ecologically relevant exposure pathways, as they are not identified as exposure pathways in the BERA conceptual site model, and will not be used to derive TRVs for the BERA.

Several specific data preprocessing questions have arisen during the development of the aquatic biota tissue TRV derivation process. Three specific questions addressed in this section are:

1. How to handle the situation where multiple toxicological endpoint LOERs are available for a single species (e.g. both survival and growth LOERs are available for rainbow trout exposed to PCBs)?
2. How to handle the situation where multiple LOERs are available for a single toxicological endpoint for a single species (e.g. three survival LOERs are available for rainbow trout exposed to PCBs)?
3. How to ensure that survival LOERs do not elevate the TRV so that it is no longer protective of the assessment endpoint of survival, reproduction and growth as evaluated with measurement endpoint data from multiple species?

Multiple toxicological endpoint LOERs for a single species: Assessment endpoints identified in the Portland Harbor BERA problem formulation are intended to be protective of survival, reproduction and growth of multiple aquatic biota groups. The most commonly measured adverse effect of bioaccumulated chemicals in aquatic species is mortality. Lethal body burdens of contaminants are generally higher than residues associated with adverse effects on reproduction or growth. Therefore, an SSD based on residue-effects data protective of all three toxic effects identified in the BERA assessment endpoints run the risk of being underprotective of reproductive and growth effects if the SSD is based largely on mortality data. To derive SSDs based only on reproduction and growth data runs the risk of severely limiting the number of tissue TRVs that can be derived, due to the relative lack of residue-effects data for reproductive and growth endpoints compared to the amount of data available from lethality studies.

The normal procedure used to derive a TRV from a SSD is to take the geometric mean of multiple toxicity studies available for a single species, then use the calculated geometric mean as the toxicity value within the SSD for that species. However, it does not appear reasonable to calculate a mean of mortality, reproductive and growth LOERs to obtain the toxicity value for a given species.

The approach to handling multiple toxicological endpoint LOERs for the same species will be to incorporate the lowest LOER of the available endpoints for each species into the final SSD. This approach will result in each species accounting for only one data point within the SSD for a given chemical. Data preprocessing methods for deriving the toxicity value for a given toxicological endpoint for a given species from multiple LOERs, and incorporation of survival data into mixed toxicological endpoint SSDs will be presented in the next two sections.

Multiple LOERs for a single toxicological endpoint for a single species: A situation often encountered is where multiple LOERs are available from different studies with the same species for the same toxicological endpoint (e.g. three LOERs are available for PCB residues affecting *Daphnia pulex* fecundity). A commonly employed approach to address this situation is to calculate the geometric mean of the multiple studies, then use the calculated geometric mean as the toxicity value for that species and endpoint within the SSD. This is the approach used by EPA during its derivation of AWQC when multiple studies of the same adverse effect are available for a species (e.g. species mean acute value), or for different species of the same genera

(e.g. genus mean acute value). For aquatic biota tissue TRV derivations for the Portland Harbor BERA, the geometric mean of multiple studies of a given species within the same toxicological endpoint will be used as the toxicity value incorporated into the SSD for that species and endpoint.

Processing of mortality LOERs: A TRV based largely or completely on mortality LOERs may not be protective of reproduction or growth. To ensure that the aquatic biota tissue TRVs for the Portland Harbor BERA are protective of all environmental attributes within the BERA assessment endpoints (i.e. survival, reproduction and growth), an uncertainty factor will be applied to the mortality LOERs before the mortality LOERs are further preprocessed and subsequently incorporated into an SSD.

Once the mortality LOER values are obtained, each mortality LOER is divided by an uncertainty factor of 8.3 to calculate the toxicity value for each species. The value of 8.3 is the mean acute-chronic ratio of 456 same-species pairs of acute and maximum acceptable toxicant concentrations for metals, narcotics, pesticides, and other organic chemicals as calculated by Raimondo et al. (2007). Within the BERA tissue TRV derivation, the factor of 8.3 is intended to convert the LR_X concentration (LR_x = lethal residue to x percent of the study organisms) to an "LR_{LOW}" value, expected to be an $LR_{<1 \text{ to } 10}$ that should result in little or no toxicity to the test species. A LR_x based on unadjusted LR_x mortality values without the 8.3 adjustment factor would be an underprotective criterion that potentially elicits toxicity to an unacceptably high proportion of the individuals of the test species. Similarly, a tissue TRV based on LOERs lethal to a substantial portion of the test organisms within a study would not be protective of the survival, reproduction and growth assessment endpoints within the Portland Harbor BERA. The 8.3 acute-chronic ratio identified by Raimondo et al. (2007) as the mean ACR of over 400 aquatic toxicity studies will be used as the uncertainty factor to be applied to all residues associated with mortality used to generate tissue TRVs.

Review Process: Once the TRVs have been derived, a final review should be made. The purpose of the review is to check the accuracy of the calculations, and to ensure the desired protectiveness of the TRVs has been attained for all receptor species. If the derived TRV is higher than an adverse effect residue from the literature for a target ecological receptor being assessed, the TRV should be reevaluated and revised downward if necessary for protection of the target receptor. This process is analogous to the "final checks" step in derivation of AWQC (Stephan et al. 1985) in which the SSD-based final acute or chronic values are compared to individual studies to see if the calculated values might have to be lowered to protect this individual species. This evaluation is particularly important for receptor species to be evaluated at the organism level. If the derived TRV is higher than an adverse effect residue from the literature for a target ecological receptor being assessed at the organism level, the TRV will be reevaluated and revised downward if necessary for protection of the target receptor. In particular, the available salmonid residue-effects data will be evaluated closely to ensure organism-level TRVs based on SSDs are adequately protective.

Aquatic Biota Tissue TRV Derivation Procedure for the Portland Harbor BERA

Studies excluded from use in deriving aquatic biota tissue TRVs in the BERA include the following:

- Endpoints were not related to effects on survival, reproduction, growth or fish behavior
- Biota were exposed to mixtures in the laboratory. Exceptions to this are certain mixtures of related chemicals such as PCB Aroclors, Clophens or other PCB mixtures; mixtures of DDT and its metabolites DDD and DDE; or mixtures of chemicals such as dioxins, furans and certain PCB congeners with dioxin-like (i.e. 2,3,7,8-TCDD) mechanisms of action where the toxicity can be expressed as toxic equivalency factors relative to the toxicity of 2,3,7,8-TCDD.
- Studies where biota were exposed to chemicals in the field. This is because effects observed in field studies generally cannot be associated with a specific chemical

The specific requirements and toxicity data preprocessing approaches to be used during the derivation of SSD-based aquatic biota tissue TRVs for use in the Portland Harbor BERA are presented below. Most of these requirements are also appropriate for use with the lowest value approach to deriving TRVs.

If the sample size is ≥5, the SSD approach will be used and if the sample size is <5 the lowest value approach will be used[1].

- TRVs to be based on lowest observed effect residue (LOER) data affecting survival, reproduction, growth or (for fish only) behaviors that can be linked reliably to survival, reproduction, or growth
- All LOERs have equal weight in the SSD (i.e. no one adverse effect such as reproduction is weighted more heavily than any other adverse effect)
- LOERs must be measured, not modeled or predicted.
  - LOERs reported in a companion study to the citation reporting adverse effects, but not in the original effects study are acceptable for use
  - LOERs described in terms of a measured bioconcentration or bioaccumulation factor from a water or dietary exposure concentration or dose are acceptable once converted into the equivalent measured residue value
- Minimum of five toxicity data points required to derive a TRV from an SSD
- 10th percentile of the LOER SSD to be used as the TRV for measurement endpoints evaluated at a population or community level of biological organization
- 5th percentile of the LOER SSD to be used as the TRV for measurement endpoints evaluated at the organism level (i.e., these TRVs would be applied to both juvenile salmonids, and lamprey ammocoetes)
- Growth and reproduction LOERs to be weighted equally, as reported from the literature, without application of any uncertainty factors

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[1] Sample size refers to the number of species with tissue-based toxicity data available that meet the requirements outlined above, not the total number of individual toxicity data points. As the intent of the SSD approach is to have only one data point for each species in each chemical SSD, the approach is a true SSD approach.

- Studies where control group data are available for comparison to treatment groups are preferred, but is not an absolute requirement for a study which reports only effects residues to be incorporated into an SSD. Studies without control groups should be noted in the data tables listing all studies used in TRV derivation.
- Studies where both adverse effect residues and the magnitude of the observed effect are statistically significantly elevated above controls are preferred, but is not an absolute requirement for a study to be incorporated into an SSD. Studies without statistical significance reported should be noted in the data tables listing all studies used in TRV derivation.
- LOER residues for mortality will be divided by an uncertainty factor of 8.3 (Raimondo et al. 2007) to convert lethal residues to residues where lethality is indistinguishable from acceptable control mortality, then weighted equally with the growth and reproduction LOERs, without application of any other uncertainty factors. Use of this default uncertainty factor will be used for all survival LOERs unless sufficient data exist to estimate chemical specific acute-chronic ratios.
- Literature citation must be the primary source of the toxicity data
- Species must be reported
- Exposure to a single contaminant only in a laboratory setting
  - Exceptions to this requirement will be made for chemicals commonly evaluated as a single chemical even though they are mixtures (e.g. PCB Aroclors or Clophens; chlordane; toxaphene, DDT and its metabolic transformation products DDD and DDE, which can be reported as total DDTs; dioxins, furans and certain PCB congeners with dioxin-like (i.e. 2,3,7,8-TCDD) mechanisms of action where the toxicity can be expressed as toxic equivalency factors relative to the toxicity of 2,3,7,8-TCDD
- Individual literature citations must report information on a minimum of two exposures concentrations or doses: one control and at least one contaminant exposure
- EPA prefers the TRVs to be presented in units of mg/kg (or µg/g), whole body wet weight. Dry weight TRVs are acceptable as long as it is clearly stated whether the units are in terms of wet or dry weight. A majority of the residue-effects literature is reported as wet weight. EPA does not believe sufficient residue-effects literature are available in a form to permit derivation of lipid normalized TRVs for organic chemicals
- Unless the water content of tissue in a citation is explicitly given, assume 80% water content of tissues when converting literature LOERs between wet and dry weights
- Beneficial effects (e.g. hormesis) will not be used to derive the TRV unless the hormetic effect can be directly related to an adverse effect on the assessment endpoints
- Adverse effects associated with nutritional deficiency of essential elements (e.g. copper, selenium, zinc) will not be used to derive TRVs
- LOER data from both freshwater and marine species may be used
- Species not required to be limited to North America residents
- Injection or gavage studies will not be used to derive tissue TRVs
- No uncertainty factors will be applied to either reproduction or growth LOERs
- If multiple LOERs are available for a chemical's toxicological effect in the same species (e.g. three growth LOERs are available for rainbow trout exposed to PCBs), the

geometric mean of the multiple LOERs will be calculated, and the calculated geometric mean used as the single toxicity value for that species and toxicological endpoint
- If multiple LOERs are available for different toxicological effects for a single species (e.g. both survival and growth LOERs are available for rainbow trout exposed to PCBs), the toxicological endpoint with the lowest LOER for that species will be incorporated into the SSD
- Aquatic plant data should not be used to derive tissue TRVs for fish and aquatic invertebrates

Hierarchy of Procedures to Develop Aquatic Biota Tissue TRVs

The hierarchy for developing aquatic biota tissue TRVs, in units of mg/kg whole body wet weight, is as follows:

1. Taxa specific TRV using a species sensitivity distribution. The availability of residue-effects data will dictate the level to which this approach can be used, but we anticipate the lowest taxon to which tissue TRVs can be developed will likely be at the level of fish TRVs or invertebrate TRVs.
2. For selenium in fish tissues, use the EPA (2004) draft fish tissue criterion. Based on the screening level ecological risk assessment results to date, no selenium in invertebrate tissue TRV is required for the Portland Harbor BERA.
3. Aquatic biota TRV applicable to all aquatic species using a species sensitivity distribution. The SSD may include data from fish, invertebrates, and larval amphibians
4. For chemicals with insufficient residue-effects data to permit development of a species sensitivity distribution, utilize existing TRVs as previously developed and proposed by LWG in various documents if the TRVs are approved by EPA.
5. For chemicals with insufficient residue-effects data to permit development of a species sensitivity distribution, and without TRVs previously derived by LWG and approved by EPA, the lowest value approach (i.e. lowest LOER) from the available literature will be used to define the TRV.
   a. If a mortality LOER divided by 8.3 (Raimondo et al. 2007) or by the species-specific acute-chronic ratio is lower than the lowest growth or reproductive LOER, the mortality LOER divided by 8.3 or by the species-specific acute-chronic ratio will define the TRV
   b. If the lowest LOER is a mortality endpoint, the mortality LOER will be divided by 8.3 (Raimondo et al. 2007) to define the TRV.

Once the TRVs have been derived, a final review is conducted to check the accuracy of the calculations, and to ensure the desired protectiveness of the TRVs has been attained for any of the receptor species. This evaluation is particularly important for receptor species to be evaluated at the organism level. For example, if the derived TRV is higher than an adverse effect residue data point on an SSD for a salmonid species, the TRV should be reevaluated and revised downward if necessary for protection of juvenile salmonids. As no residue-effect studies are available for any lamprey species, this type of review will not be possible to ensure the protectiveness of the tissue TRVs for lamprey. The absence of any lamprey residue-effects

literature against which tissue TRV protectiveness can be evaluated is an uncertainty in the BERA.

Chemicals for Which Aquatic Biota Tissue TRVs Need to be Derived

The EPA produced screening level ecological risk assessment (SLERA) for Portland Harbor as part of its review of the LWG's Round 2 Report identified 17 tissue COPCs for fish species (no fish species contained residues exceeding all 17 identified COPCs), and 23 tissue COPCs for aquatic invertebrates (again, no one invertebrate receptor contained residues exceeding all 23 identified COPCs). Ten of the COPCs were common to both fish and invertebrates, so the maximum number of COPCs for which aquatic biota tissue TRVs need to be derived, based on the results of the SLERA, is 29.

Of these 29 chemicals, seven are various PAH compounds for which Shephard (1998) concluded generally applicable tissue TRVs should not be derived. This conclusion is based in part because of the rapid metabolic transformation and/or photoactivation of parent PAH compounds to more toxic metabolites, whose toxicity is not properly evaluated by tissue benchmarks for a less toxic parent compound. Also a factor arguing against derivation of tissue TRVs for PAHs in this BERA are observations that the metabolic transformation abilities differ among species, and the transformation ability has no clear relationship with taxonomy (i.e. although a common presumption is that fish more actively transform PAHs than do invertebrates, many invertebrate species are better able to transform PAHs than some fish species). Among the freshwater invertebrate species able to metabolically transform PAHs are crayfish (Jewell et al. 1997), fingernail clams and *Chironomus riparius* (Borchert et al. 1997).

The tissue TRVs required for the Portland Harbor BERA are listed below.

Fish and invertebrates	Fish only	Invertebrates only
Zinc	Chromium	Antimony
Total PCBs	Lead	Arsenic
4,4'-DDD	Mercury	Cadmium
4,4'-DDE	Selenium	Copper
4,4'-DDT	δ-hexachlorocyclohexane	Tributyltin
Total DDX	Hexachlorobutadiene	Endrin
β-hexachlorocyclohexane	Butylbenzyl phthalate	
Lindane		
Bis(2-ethylhexyl)phthalate		
Di-n-butyl phthalate		

The above lists are the chemicals of potential concern (COPC) identified during the screening level ecological risk assessment. The COPC list is based on the results of Portland Harbor sampling up through the end of Round 2 data collections, as determined by LWG's evaluation in their Round 2 report and EPA's review of the Round 2 report. Round 3 tissue data will have to go through the same screening process as the other site data has already gone through. The possibility exists, therefore, that additional chemicals detected in the Round 3 tissue samples will require derivation of tissue TRVs for the BERA.

Calculation Procedures for SSD Derived Aquatic Biota Tissue TRVs

In order to permit verification of the calculated TRVs once the toxicity data to be incorporated into each SSD has been compiled, EPA recommends that software be used that is freely available to all interested parties, making it possible for all to confirm the TRV calculations, while meeting the need for estimation of both 5th percentile and 10th percentile TRV derivations. One such program that is freely available and specifically designed to fit toxicity data to species sensitivity distributions is the BurrliOZ software from the Australian Commonwealth Scientific and Industrial Research Organisation (CSIRO). BurrliOZ shows the fit of toxicity data to Burr Type 3, log-logistic and lognormal distributions. BurrliOZ also calculates both the effect concentration at a user defined percentile of the SSD, and the percentile of the SSD for a user defined environmental concentration. The software may be freely downloaded from the following web site: http://www.cmis.csiro.au/envir/Burrlioz/

A second free and publically available program that can be used to estimate percentiles of an SSD is the ET_x software (van Vlaardingen et al. 2004) developed by the Netherlands National Institute for Public Health and the Environment (RIVM). ET_x uses a log-logistic distribution to fit data to an SSD, and also estimates confidence limits around the selected effects percentile. The software may be freely downloaded from the following web site:
http://www.rivm.nl/rvs/overige/risbeoor/Modellen/ETX.jsp

The ETx version 2.0 manual can be downloaded from the following web site:
http://www.rivm.nl/bibliotheek/rapporten/601501028.html

Specific selection of the SSD model to be used will be based on which best fit the actual distribution of data for a given chemical. This "best fit" selection approach is becoming increasingly used for derivation of SSD-based environmental criteria worldwide, and so is appropriate for use in the Portland Harbor BERA.

Calculation Procedures for Lowest Value Approach Derived Aquatic Biota Tissue TRVs

The lowest species mean LOER will be used as the TRV for sample sizes <5. The species mean LOER will be calculated as the geometric mean LOER for a given species. The species mean LOER will be based on the most sensitive endpoint of the available data for that species. For example, if both growth and mortality toxicity data are available for a species, and growth is a more sensitive endpoint than mortality, then the species mean LOER will only be calculated from the growth-based toxicity data. Thus, the tissue TRV from the lowest value approach will reflect the most sensitive endpoint for the most sensitive species. It should be noted that the lowest value approach can be less conservative than the SSD approach for moderate sample sizes, which can result in 5th or 10th percentiles lower than the lowest toxicity value. This can result in a TRV that is less conservative for the chemical with the smaller sample size (i.e., too small to use a SSD). This uncertainty will need to be addressed in the Uncertainty Analysis section of the BERA, and incorporated accordingly into chemical-specific implementation of the weight of evidence scheme for any tissue-based line of evidence.

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